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Regulation of vascular morphogenesis by the matricellular protein SPARC

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Activated endothelial cells are capable of assembling three-dimensional multicellular structures known as capillary beds in response to several different classes of regulatory macromolecules. Understanding the regulatory pathways that control growth and regression of new blood vessels is a major goal of researchers in vascular biology today. Regulation of vascular morphogenesis in endothelial cells can be exerted at the levels of cell-cell adhesion, cell-matrix interaction, and control of the cell cycle. The process of angiogenesis, that is, the sprouting of new vessels from a parent microvessel, encompasses various sequential but often coincident stages (Fig. 1). Both angiogenic and anti-angiogenic factors, some of which are listed in Table 1, are believed to target one or more of the stages [1–6]. Thus, regulation of angiogenesis is a dynamic process determined by the balance between counteracting regulatory factors.

The most prominent positive regulators of angiogenesis, vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) and basic fibroblast growth factor (bFGF or FGF2), signal directly to endothelial cells through their specific receptors, whereas others (PDGF, TGF-β, and IGF-1) appear to involve indirect mechanisms [1, 2, 7–9]. The mode of action of angiogenesis inhibitors includes cell cycle arrest (platelet factor-4) [10], inhibition of signal transduction by growth factors (16 kDa prolactin) [11], and/or abrogation of bFGF signaling by truncated high-affinity receptors for bFGF in the circulation and in the basement membrane of some endothelial cells [12]. Recently, a newly-defined class of regulatory macromolecules known as matricellular proteins has received considerable attention, since two of its members (SPARC and thrombospondin 1) have been shown to modulate angiogenesis [6, 13–15]. By definition, these secreted proteins interact with cell-surface receptors, growth factors, and matrix proteins but do not function as structural matrix components. One of the hallmarks of glycoproteins belonging to this group is their ability to disrupt cell-matrix interactions [16, 17]. SPARC can be considered as a prototype for this class of counteradhesive proteins that appear to play dynamic roles in tissue remodeling during normal development or in response to injury.

SPARC (secreted protein acidic and rich in cysteine), also known as BM-40, osteonectin, and 43 K protein, is comprised of four distinct structural domains based on the predicted secondary structure (Fig. 2) [14, 16, 17]. Of particular interest is our finding that a highly conserved, plasmin-sensitive, copper-binding motif Lys-Gly-His-Lys (KGHK) of SPARC domain II stimulated angiogenesis in the chick chorioallantoic membrane (CAM) assay in a concentration-dependent manner (Fig. 3), whereas intact SPARC displayed a null or anti-angiogenic activity [18, 19]. Consistent with this finding is the spatial and temporal restriction of SPARC expression to sites of growing capillaries in the CAM (Fig. 4), with coincident increases in SPARC proteolysis [19]. In contrast to the growth-promoting properties of the KGHK motif, a cationic region of domain II and a Ca^{+2}-binding EF-hand region of domain IV mimicked the activity of native SPARC and inhibited DNA synthesis by the arrest of endothelial cells in the G1 phase of the cell cycle [20–22].

SPARC can also regulate cell proliferation indirectly by binding to cytokines. It was shown to interact specifically with the PDGF-B-chain and abrogate binding of PDGF-AB and -BB to their receptors on fibroblasts [23]. Through an apparently different mechanism, SPARC inhibits the mitogenic and chemotactic effects of bFGF on bovine aortic endothelial cells *in vitro* [24]. Our recent results suggest that VEGF-induced proliferation of bovine and human endothelial cells *in vitro* can be inhibited by SPARC and SPARC peptides from domains II and IV in a concentration-dependent manner by specific binding to VEGF and competition of the binding of VEGF to its receptors (C. Kupprion, E.H. Sage, manuscript in preparation). Whether interaction of SPARC with VEGF can affect vascular morphogenesis is currently being addressed in our laboratory by quantification of the effect of SPARC on VEGF-stimulated angiogenesis (P. Parsons-Wingerter, C. Kupprion, and E.H. Sage, manuscript in preparation).

As a counteradhesive protein, SPARC mediates cell shape changes by disrupting focal adhesions, redistributing actin filaments, inhibiting cell spreading, and inducing cell rounding [14,

Table 1. Proteins and peptides regulating angiogenesis

Angiogenic factors	Anti-angiogenic factors
SPARC (KGHK motif)	SPARC (domains II and IV)
Platelet-derived growth factor	Angiostatin
Vascular endothelial growth factor	Platelet factor 4
Acidic and basic fibroblast growth factor	16 kDa prolactin
Insulin-like growth factor	Thrombospondin-1
Interleukin 8	Interleukin 12
Hepatocyte growth factor (scatter factor)	Interferon α, β
Transforming growth factor-β1	

Information in this table is reviewed [1–6].

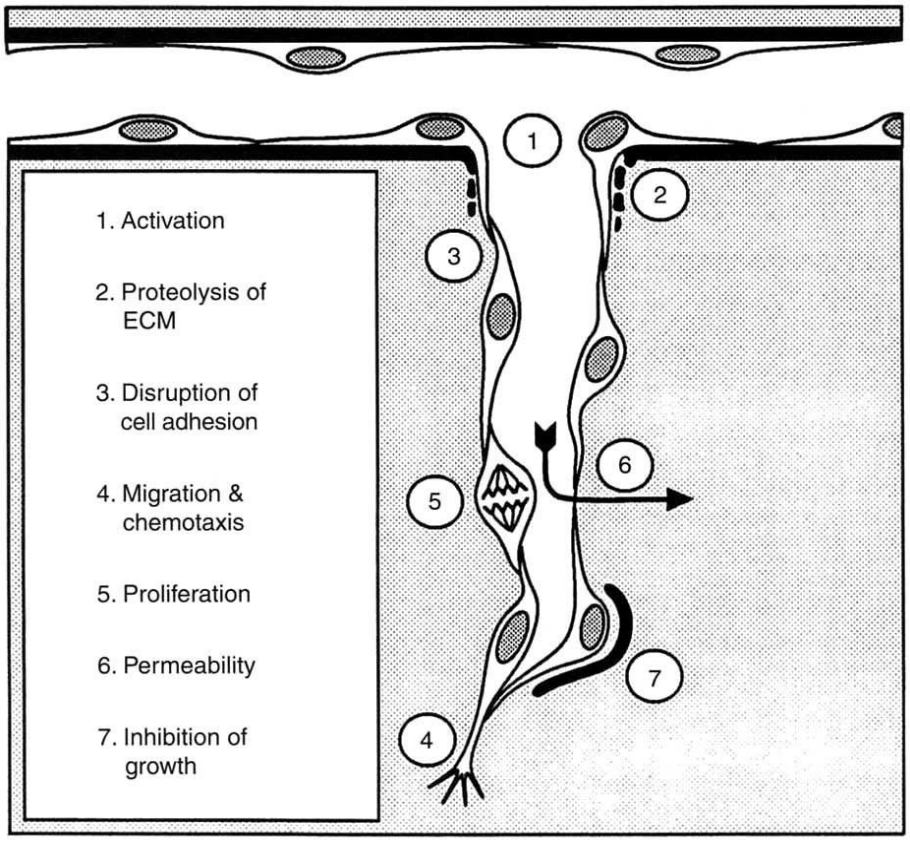

Fig. 1. *The process of angiogenesis.* The various stages of the formation of a capillary sprout from a pre-existing microvessel are numbered. These stages are not necessarily sequential. Basement membrane is shown as a thick black line, and the interstitium is the stippled area surrounding the sprout (Drawing provided by R. Vernon, University of Washington, and partially adapted from [5]. Reprinted with permission from *Advances in Oncology*, 12(2): 17–29, 1996 and Cliggott Publishing Co.).

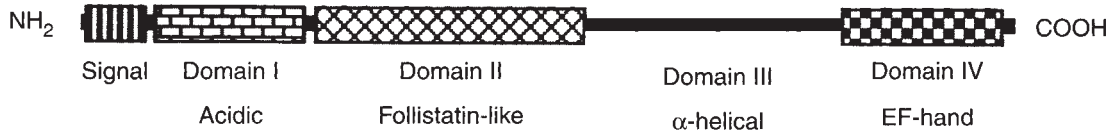

Fig. 2. *Schematic representation of the murine SPARC protein.* Structural domains are highlighted by boxes. Collagen IV-binding domain III and high-affinity calcium-binding domain IV were recently shown to form a single autonomously folding and crystallizable module known as the EC (extracellular calcium-binding) domain [29].

Fig. 3. *Concentration dependency of the angiogenic activity of the KGHK motif.* Vitrogen (native type I collagen) was cast into meshes with different concentrations of KGHK, and applied onto CAMs. After 24 hours, the preparations were removed, fixed, and sectioned. Transverse sections of the mesh were stained with hematoxylin and eosin. Blood vessels are are indicated by arrows. Asterisks depict openings in the nylon mesh. (**A**) Vitrogen and PBS; (**B**) KGHK, 0.01 mM; (**C**) KGHK, 0.5 mM; KGHK, 5 mM. Bar = 100 μM. (Reprinted with permission from *Molecular and Cellular Biology*, 6:327–343, 1995, and Cliggott Publishing Co., [19]).

Fig. 4. *Distribution of SPARC in the CAM.* (**A**) Camera lucida illustration of the vasculature in a day 10 CAM is divided into three zones that correspond to regions of developmental age. Zone I contains the largest and developmentally oldest vessels. Zone III consists of the highest percentage of actively growing vessels in the periphery. Zone II corresponds to the transitional region between the other two zones [19]. (**B**) Confocal analysis of a day 10 CAM whole mount after staining with anti-SPARC peptide antibodies followed by FITC-conjugated secondary antibodies. Fluorescence intensity is translated into a grey scale shown on the left. Highest concentrations of bound antibody are shown in red (+), and lowest concentrations are shown in violet (−). Arrows indicate areas with high levels of expression of SPARC. (This figure is reprinted from a color graphic in [19]; used with permission from *Molecular and Cellular Biology*, 6:327–343, 1995, and Cliggott Publishing Co.).

17, 25, 26]. Moreover, addition of SPARC to endothelial monolayers in culture exhibiting tight cell-cell appositions without intercellular gaps resulted in a time-and concentration-dependent increase in the transendothelial flux of ^{14}C-BSA [28]. This effect of SPARC was coincident with the redistribution of actin-containing stress fibers since pretreatment of endothelial cell monolayers with F-actin stabilizing agents protected against this SPARC-induced barrier dysfunction.

The spatial and temporal restriction of SPARC expression during tissue remodeling, embryogenesis, and in response to injury indicates an important role for this protein in normal development [14, 30–33]. Microinjection of *Xenopus* embryos

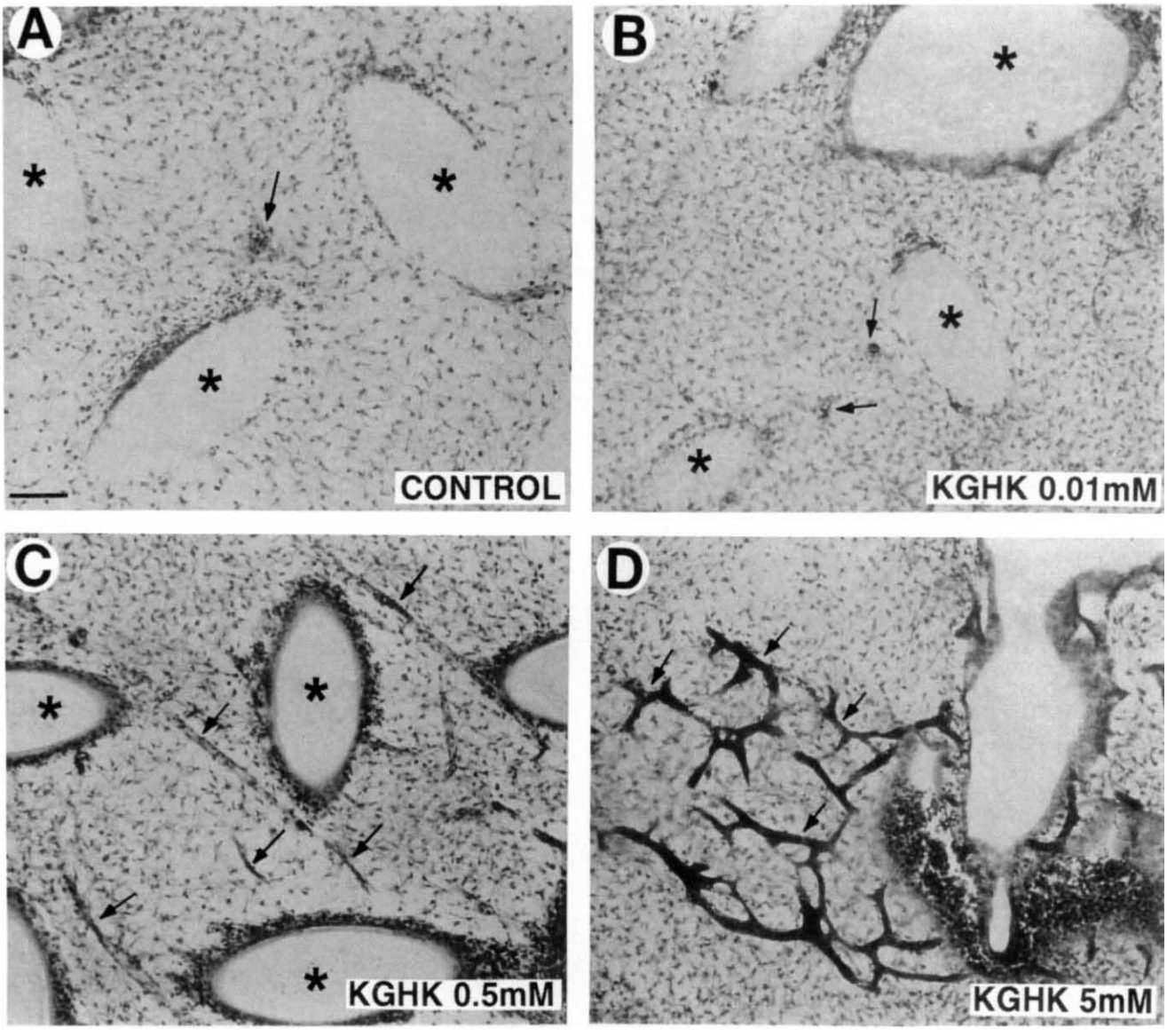

Fig. 3.

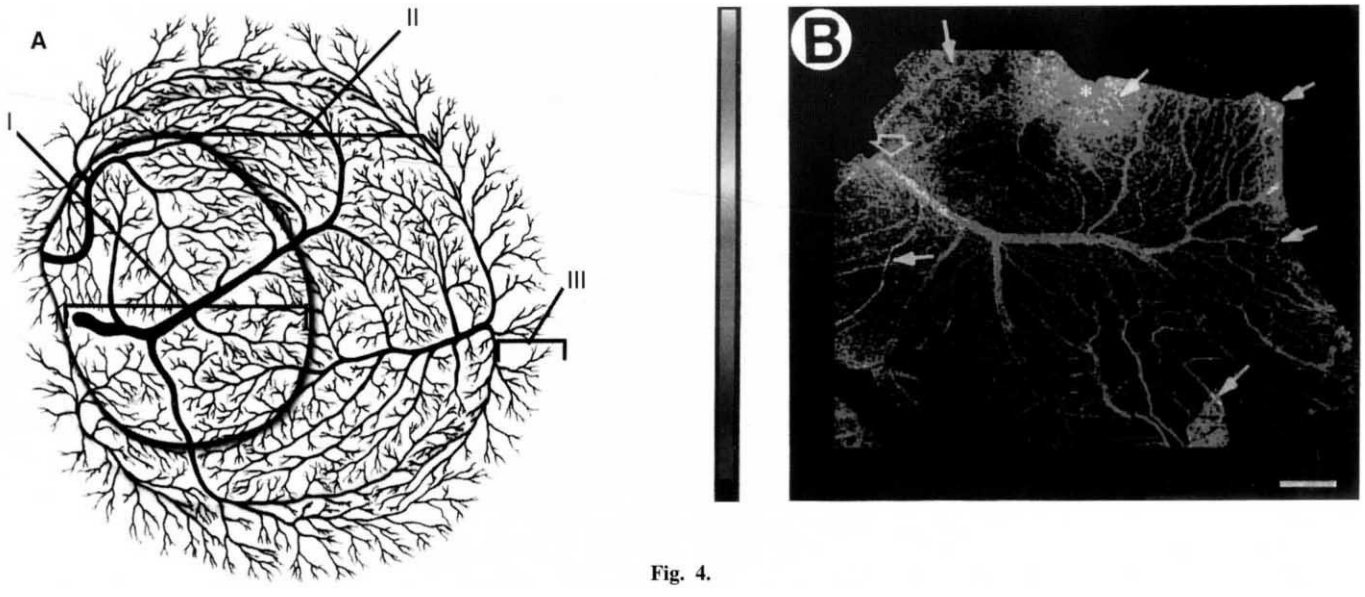

Fig. 4.

with antisense SPARC mRNA resulted in developmental defects [34]. Overexpression of SPARC in muscle cells of *C. elegans* resulted in an "uncoordinated" (Unc) phenotype, in which adults moved abnormally or became paralyzed. In mice, the elevated production of SPARC found in the gut, bone, and gonads, is suggestive of a role in tissue remodeling, cell movement, and/or proliferation in certain organs [30, 32]. Recent ablation of SPARC by gene targeting has resulted in viable animals that exhibit a slower rate of dermal wound healing (C.C. Howe, P.W. Soballe, A. Basu, A. Cesano, M.W. Xu, E.H. Sage, L.D. Thornburgh, S. Samulewicz, unpublished observations) [35]. These results support a role for SPARC in modulation of cell-matrix interactions, cell attachment, and/or synthesis and turnover of extracellular matrix.

One of the most fundamental questions to be answered about SPARC biology is: through which signal transduction pathway(s) does SPARC mediate its counteradhesive and anti-proliferative functions? Recent studies on cultured bovine endothelial cells indicate that SPARC acts initially as a counteradhesive protein through tyrosine phosphorylation-dependent pathways [36, 37]. Inhibitors of protein tyrosine kinases appear to protect against the SPARC-induced changes in barrier function, actin rearrangement, and changes in cell shape [36, 37]. Inhibition of cell cycle by SPARC is believed to be a more distal effect and does not appear to involve a tyrosine phosphorylation-dependent pathway [37; K. Motamed, E.H. Sage, unpublished data]. Isolation of cell surface receptor(s) for SPARC will undoubtedly provide a more precise understanding of the exact mode of signaling by this matricellular protein and help us achieve a more accurate understanding of its involvement in specific stages of vascular development.

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